rRNA Gene Cluster

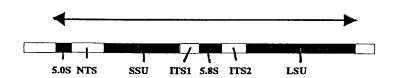


FIG. 1

5 S

_				
1				50
AAAGTCGCAC	CTTTCCCCAT	AAACCCCCTC	CCCACCCCCT	TGGACATTGT
51				100
TCCACTTTTC	ACTTGTATTG	TGAAGCACCC	AATGCTAGCC	CATAGAACAG
101				150
TCCAGTAGTT	CAATAGAGAG	ACTAGTGAAC	ATAGTTTATA	ACATTGTCCA
151				200
AGGGGTGGAG	GGGGATGCGC	GAAATCGATG	TGCACGTTTG	GTCAAAGATG
201				250
CTCGCGAAAG	CTGCACATCA	ATTTCGCACA	TGGGCGAAAT	TGACTTGCAG
251				300
GTGGGTATAA	AAGTTGATGT	AGGCCATGTG	GCTCGATTTC	AACCATATGG
301				350
GTATGCTTCT	GAGGATGGGG	TGTTACAGTG	GACCATATGA	GGTAGGTCAT
351				400
TTGGAGATGT	CACCAAAATG	GTCTAAATCT	GCGCATTCCA	TTTAAGTGAA
401				450
TTTAAGTGAA	ATTTAAGTGA	ATTTTACTTA	AAATTGACCT	TTTTCGTTGC
451				500
GCAGATTTGG	GGTGGTGATG	GGTGACGCGG	CGAATTTTTT	AAAAAAGAGG
500				550
TATATCGCGT	GCTATTTGTA	TTTTTGGTAT	CACCGCGTCA	CCAATCACCA
551				600
TTGACGGTTT	CTTTTTCGAA	GTTTTTCCGG	ATTATTGCAT	TTTTTATATA
600				650
ATTGTGGGTG	GCTGATTCTT	GCGAAAGGAC	TGTTGTGATG	TCCGAGTTCC
651				700
CAAATTGGGA	GTTTTTGGAC	ATCACTCCTG	ATCTGCCGGC	GGCGATCAGG
700				750
ATGACTGACA	TTTCGATATA	TTTTGGGTAT	TCGATAGCTG	CCAAATCGGT
751				800
CAGCGTCGAG	TATTCCGGTT	TATTCGAAGG	ATTCATGATA	TTGCAAAATA
800				850
TCATTGATTT	TCATGGGGTT	TTGTATTAGT	ACCCGCTCAT	TGTGGGAAAG
851				900
TCGGGTGGAT	TTATCTTACC	CGCAAATCTA	ATACAAGATT	TGCATGATGC
900				950
AGCAATAGAC	CAAGGTTAGT	ATAGCAGTTG	TATTTATACG	ACTAGTTATG
951				1000
CAAACCCTTT	GTGTTTTTTG	TTGCGACTCT	TGGCGTGAAC	CGGAAGACCG
1000				1050
GACCTCGCTT	TCGACTATTC	ATCTTTGATG	GATATGAGAT	CGCAAGGGTA
1051				1100
TCGCTTCGTG	CGATATTTAG	TGACCATCAG	AGCACGCTAC	GACTTTTGAT
1100				1150
TATATCCTTG	GATTTAATCG	GAAGCTCGCA	AGCATTGCAT	TGATGCAATC

FIG. 2

#1501 -- -

ttttcaTTTT TGCTTTCACA ACCCCGCACC CCATGTACAA TGTTGCCAAC #1 CACTAGAGTT TCAACAACAT TCGGATTTGA CAACATGTCA ACAATTCACA ACAGAAATTG ACAACATTGT CACAAATTCT CAAATTGGAC AACATTGGAC #101 AAAAATTCAC AACATACATT GGACAACAGT GGACAACGAA CCCAAACCCG #151 ACAACATTGT CCAGGGGGAT AGGGGGTGAA AAAGCAGTGC CGGCAAAGTC #201 GAAAGATGTC AAGTTGGAAT GCGGCTCAAA TTCGTCATTT GTGTAAATCC #251 GCAATTTTGC CAATGTGCAA TTTTGCAAAT GTGCAATTTT GCAAATGTGC #301 AATTTTGCCA ATGTGCAATT TTGCAAATGC GCAATTTTGC AAATCCGCAA #351 TTTTGCAAAT GTGCAATTTT GGAAAATCAC CAAATGAAAA TCGTCCAAGT #401 CGAATTGGAG GCGTGGTGAC ATGGTCCCGG GATCCCCTGG TTACAGTGGA #451 CAATATCCCA GCAATATTCG CTGTAATTTG GAGTTTCGCT GTTTTGGCAA #501 ATTTTGAGTC TGAAAAAAA AATTGCAAAT GCGCAAAGGG GGTGAAGGAA #551 AAAAAAGCAC CCCCGAAGGT AAAATTCCCT TTAAGTCCCT TGCGCATTTG #601 CAAAATTTTC AAAAATTGTT GCAAATGCGC TTTTGTTATT TGGCCGGTTC #651 ATTGGTGTCA AAAGTTGCCT GGGGTGGTTA CACAATGCAC GGAATTGGTT: #701 GGAAGTTGTG TGATTGAAAA TTGGTCGTGT CACACAATTT TGCGCATTTG CAAAAATTCG CAAATTGGAC AAAAAAGGGT CGCGCACAGT CAAATTGCGC AAATTTCACT TTGAAGTGAG TGCGCATTTG TGGGGCAGAA ATGTGGTGAC #851 AGCATCGTTT TTTATAATAA ATATTCTATA TTTAGTATCT TTATTATAAT #901 TTGCTGTCAC CAATCACCAT TTTAGAATTT TTATTTTTTT ATGTTTTAGT #951 GACCGCGGGA TTTTTTGCAA AGTACTATYG TGATGTTTGA GTTGTTTGAA #1001 ATGGGCAATT TAGAACATCA TCAGAAATCG CTGAATAGTG ATTTTTGAGT #1051 TTGACTGTTT GAAGTGTTTT GGGTATTCGG CAGCTGCCAA ATCGGTCAGC #1101 GTCGAATATA ATAGCATTTT TGTGTGTATA TGATATTTAG CGATATCATT #1151 GGAATCATGG GGTTTTGTAT TAGTACCCGC TCATTGTGGG AATGTCGGGT #1201 GGTTCAATAT CACCTGCAAA TTTAATACAG GATTTGCATG ATGCAGCGAC #1251 TGACCGGGGT TGGTATAATA GCTGATTATT CGGCTTATTA TGCAGACCTA #1301 TCGTGTTAGT AGTTGCGACT CTTGGCGTGA ACCGGAAGAC CGGAACTTGA #1351 ATTCGACTAT TTACGTCCGT AAACAGGAGA TTTCAAGAAT ATTGCACATT #1401 TTGCGTGATA TAAACGTGAT CATCTGAGCA CGCTTCGACT CTTGGATATC TGCTAATCAG CCGTCATCTG AGAGCTCGCA AGCATTGCAA TTGATGCAAT

FIG. 3

1				50
CGTGCCCTTT	TCACGAATTC	ACAGCCCCGC	ACCCCATGTA	CAATGTTGCC
51				100
CACCCGAAAT	GCCTGCCTGC	CCACCCGAAA	TGCCCGAAAT	GCCCGTTAGA
101				150
AAAAGTATGC	GAAAAGTTCT	TGTCAATTTT	GACAGTGTGT	GAAAAAACTG
151				200
AAAAAGTCCA	CTCAACATTG	CATTATGCAA	TTTGCCACTC	AACATTGTCC
201				250
AGGGGGATAG	GGGGTGAAAA	AGTATCGCAG	TCCAACTGAA	AAGATGCTAA
251				300
GTTGAAATGC	GGCGCAAATT	CATCACTTGA	GTTGCGAAAA	TCCCTAAAGT
301				350
CGAATTTGGC	ACTCGGTGAC	ATGATCGGGA	ATTTCCCTGG	TTACAGTGGT
351				400
CAAATCCCAG	CAATTTTGGC	AAAGTTTTTG	AGTTTCGCAC	TTTTCGCAAA
401				450
TTTCGTGTCT	GAAAAAAAA	TTTCAACTTT	GCGCAAAGGG	GTCAAAGGGA
451				500
AAAAAAGCAC	CCTCAAAAGG	AAATTTCCCT	TTAATCCCCT	TTGAAAAAAA
500				550
TGCGCAAAGT	TAAATTTGCG	AAAATTTCGA	TTTTCTCATA	TGACCGATTA
551				600
GTTGGTGCCA	GATGGTAGTC	GGGATGGTTA	CACGGTGCAC	GGAACTCGTT
600				650
GGAAGTTCTG	GAGTTACGAA	TTGGTCCCGT	CACCACAATT	TGCGCATTTT
651				700
TGAAATTGCG	CAAATTTGCG	AAAAAAGCAG	CGCGCAAAGT	TAAATTGTGC
700				750
GAAAATTGAC	TTTCAGGTCG	GTGCGCAAAT	TTGGGGTGAA	AAAGTGGTGA
751				800
	ATTATAATAA	ATAATCTATA	ATCTAGTTCT	TTTATTATAA
800				850
TTAGCTGTCA	CCAATCACCA	TTTGAGATTT	TTTATTTTT	TATGTTTTAG
851				900
TGACCGCGGT	ATTTTTTCCA	GAGTACTATC	GTGATGTCTG	AGTTGTCTAA
900			10001100	950
AACGGCAATT	TCAGAACATT	ACCAGAAAAC	ACTGAATAGT	GGTTTCTGAG 1000
951	ma a a amamm	maaama mmaa	GCAGCTGCCA	ATTCGGTCAG
TCTGACTGTT	TGAAGTGTTT	TGGGTATTCG	GCAGCTGCCA	1050
1000	ACTAACATTT	CIIICIICIICIII III	ATGGTATTTA	
GGTTGAATAT	ACTAACATTT	CIGIGIGIAI	AIGGIAIIIA	1100
1051		TTAGTACCCG	CTC A TOTCTCC	
1100	GGGIIIIGIA	ITAGIACCCG	CICMITOTO	1150
	ጥር እ ር ር ጥር ር እ እ	ATTTAATACA	CCATTTCCAT	
1151	ICACCIGCAA	ATTIANTACA	GONTTIGENT	1200
	ጥጥልርጥልጥልልጥ	AGCTGATTAT	ጥሮርርርርጥጥልጥጥ	
1200	1111011111111		10000111111	1250
	TAGTTGCGAC	TCTTGGCGTG	AACCGGAAGA	
1251	2			1300
	TTTACGTCCG	TAACACGTCC	GTAAACAGGA	GATTTCAAGA
1300				1350
	TTTTGTGTGA	TATAATCGTG	ATCATCTGAG	CACGCTTCGA
1351				1400
	TTTGTTAAAC	AACCGATATT	CGGGAGCTCG	CAAGCATTGC
1400				1450
AATTGATGCA	ATC			

FIG. 4

Primer	Sequence	Target
300 F	5'-CACTTGTATTGTGAAGCACCC-3'	
300 R	5'-TTG GTG ACA TCT CCA AAT GAC-3'	Perkinsus marinus
500 F	5'-ATGCTAGCCCATAGAACAGT-3'	i erkirisus mannus
500 R	5'-ATGCTAGCCCACATCACAGC-3'	
NTS7	5'-AAGTCGAATTGGAGGCGTGGTGAC-3'	
NTS6	5'-ATTGTGTAACCACCCCAGGC-3'	Perkinsus andrewsi
PM5	5'-ATGCTAGCCC ATAGAACAGT-3'	P. marinus type l
PM7	5'-CAT CTC CAA ATG ACC TAC CT-3'	P. marinus type l
PM6	5'-ATGCTAGCCC ACATCACAGC-3'	P. marinus type II
PM8	5"-CAT CTC CAA ATG ACC TAC CA-3'	P. marinus type II

FIG. 5

FIG. 6

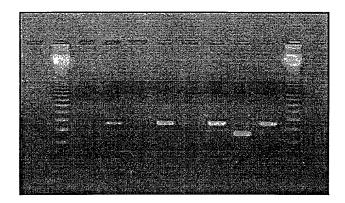
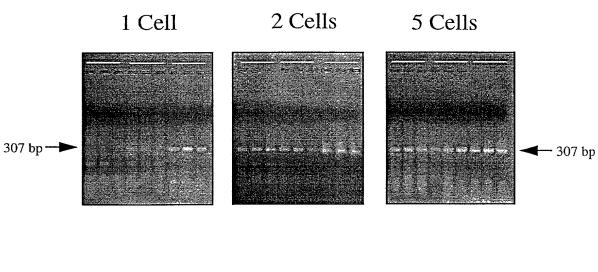
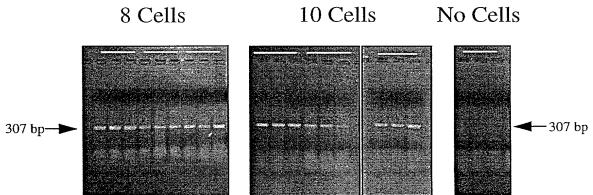
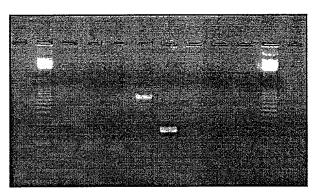


FIG. 7





Samples



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FIG. 9

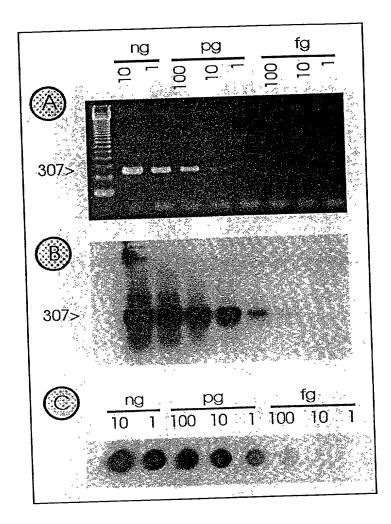


FIG. 10

	1				50	
Type-I	CACTTGTATT	GTGAAGCACC	CAATGCTAGC	CCA T A GA ACA	GTCCAGTAGT	
Type-II	CACTTGTATT	GTGAAGCACC	CAATGCTAGC	CCA C A TC ACA	GCCCAGTAGT	
	51				100	
Type-I			CATAGTTTAT			
Type-II	TCAATAGAGA	GAC G AGTGAA	CATAGTTTAT	AACATTGTCC	AAGGGGTGGA	
	101				150	
Type-I			GTGCACGTTT			
Type-II	GGGGGATGCG	CGAAATCGAT	GTGCACGTTT	GGTCAAAGAT	GCTCGCGAAA	
	151				0.00	
. -	151		1 maaaaaa 1 1 1	mmaa ammaaa	200	
	GCTGCACATC					
Type-II	GCTGCACATC	AA'I"I"I'CGCAC	ATGGGCGAAA	TTGACTTGCA	GGTGGGTATA	
	201				250	
Type-I	AAAGTTGATG	M A C C C C A TI C TI	CCCMCCAMM	CAACCAMAMC		
	AAAGTTGATG					
rybe-rr	AAAGIIGAIG	IAGGCCAIGI	GGCTCGATTI	CAACCATAIG	GGIAIGCTTC	
	251				300	
Type-I	TGAGGATGGG	CTCTTACACT	CGACCATATG	A GGTAGGTCA		
Type-II			GGACCATATG			
TYPC II	102100211000	GIGITACICI	COMECHINA	200111001011	IIIOMOAIG	
	301					
Type-I	TCACCAA					
Type-II	TCACCAA					

FIG. 11

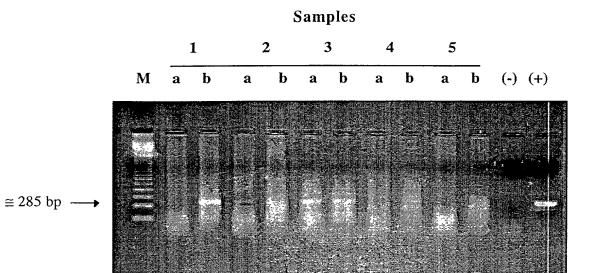


FIG. 12

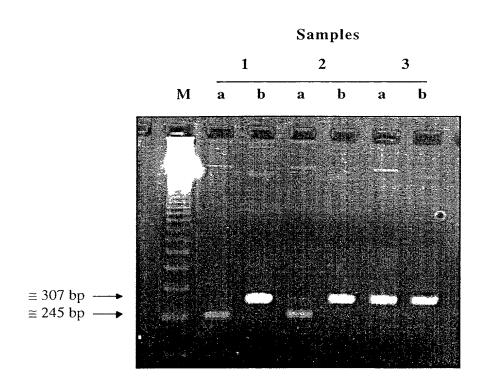
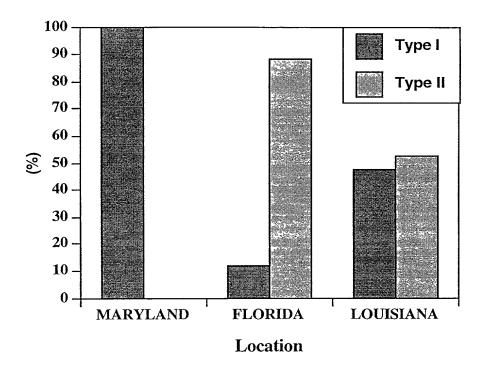


FIG. 13



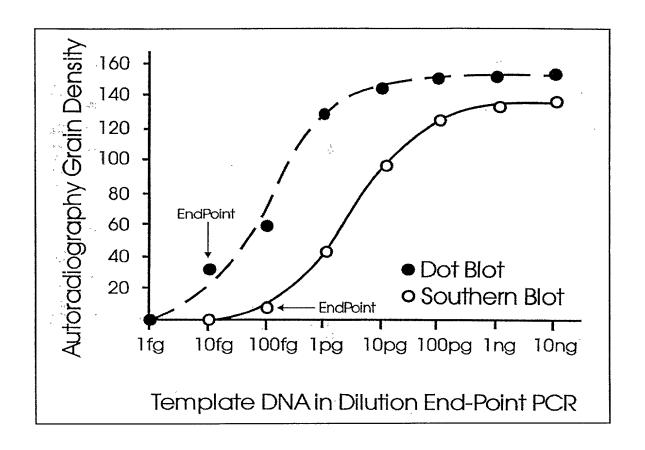
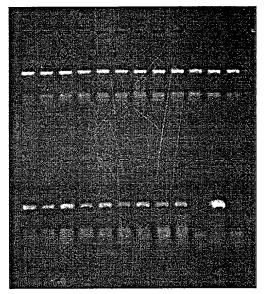


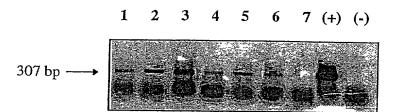
FIG. 15

Samples

1 2 3 4 5 6 7 8 9 10 11 12



13 14 15 16 17 18 19 20 1 - + -



#1					A IGCGIGCANG	
\n	a+1 a+4					
#5					A CGAGCAACAG	
>P. #1					T CTCTACAACA	
>P. #1					C AGCAGAGGGA	
.,	atlanticus	.AGTTTTGCTG	CACCCCAAC	r TTGCGCACT	r ggcgaagttg	ACTTGCAGGG
>P.	atlanticus	.GAGGGTAAAA	GATGCTATGO	TTGGTTGCG	G ACCAAGTTCG	
#25	590F-Text 51			TTGGTTGCG	ACC	
>P. #30					ACTAGTTTTT	
>P. #35					GCGCACGGGG	
>P. #40					A AAATTCACGT	
>P. #45					A AAATTTTAAA	
>P. #50					CCGTGTCACC	
>P. #55					TTCCGGAAAA	
>P. #60					AAAGGACTGT	
>P. #65					GCTCTGAAAT	
>P. #70					TGGGTATTTG	
>P. #75					CGAAGGATAT	
	1-Text				ATTAGTACCC TAGTACCC	GCTCATTGTG
 >P. a	atlanticus.	GGAAAGTCGG G			AATCTAATAC	
#85	l-Text l			• • • • • • • • •	• • • • • • • • • • • • • • • • • • • •	••••
	OR-Text				CAGCTGTTCT A	ACGGCTTGCT
>Р. а	atlanticus.	ACGCAGACCT A			TCTTGGCGTG A	
PA69 951	90R-Text	AC ········	• • • • • • • •			
>P. a	tlanticus. 1	CCGGACCTCG C	TTTCGACTA	TTCATTCCGA	TGAATATGAG A	ATTGCAAGGG
	tlanticus.	TATCGCTTCG T	GCGATATTT	AGTGATCATC	AGAGCACGCT A	ACGACTTCAG
<per2< th=""><th>-Text</th><th></th><th></th><th>AGCTCGCAAG</th><th></th><th></th></per2<>	-Text			AGCTCGCAAG		
#110	1					

>P. #1	andrewsi-S		TCCTGCCAGT	AGTCATATGC	TTGTCTCAAA	GATTAAGCCA
>P. #51					AAACTGCGAA	
>P. #10					ACTATTTGGA	
>P. #15					CGACTTCGGA	
>P. #20					CTAGTCCTTG	
>P. #25					CGGCGATGGA	
>P. #30					ATTGGCCTAC	
>P. #35					GGAGAGGGAG	
>P. #40					CGCAAATTAC	
>P. #45					ACAGGGCAAT	
>P. #50					ACGAGTATCA	
>P. #55					AGCTCCAATA	
	J3F-Text			AGTTG	GATTTCTGCC GATTTCTGCC	TTGGGCG
>P. #65					CCAGGTTTGA	
>P. #70					GTGCGCTGAC	
>P.	andrewsi-S.	GACTTTTACT	TTGAGGAAAT	TAGAGTGTTT	CAAGCAGGCT	TATGCCGTGA
#75	1					
>P. #80					TTTGGTCATA	
>P. #85					CAGTCGGGGG	
>P. #90					TTAAAGACGA	

FIG.18A

>P. andrewsi- #951	S.AAGCATTTGC CAAGGATGTT TTCATTGATC AAGAACGAAA GTTAGGGGAT
>P. andrewsi- #1001	S.CGAAGACGAT CAGATACCGT CCTAGTCTTA ACCATAAACT ATGCCGACTA
>P. andrewsi-9 #1051	S.GGGATTGGGA GTCGTTAATT TTAGACGCTC TCAGCACCTC GTGAGAAATC
>P. andrewsi-9	S.AAAGTCTTTG GGTTCCGGGG GGAGTATGGT CGCAAGGCTG AAACTTAAAG
>P. andrewsi-9 #1151	GAATTGACGG AAGGGCACCA CCAGGAGTGG AGCCTGCGGC TTAATTTGAT
<pre>>P. andrewsi-9 >SSU4F-Text #1201</pre>	S.TCAACACGGG AAAACTCACC AGGTCCAGAC ATAGGAAGGA TTGACAGATT ACC AGGTCCAGAC ATAGGAAGG
>P. andrewsi-8 #1251	S.GATAGCTCTT TCTTGATTCT ATGGGTGGTG GTGCATGGCC GTTCTTAGTT
>P. andrewsi-9 #1301	G.GGTGGAGTGA TTTGTCTGGT TAATTCCGTT AACGAACGAG ACCTTAACCT
>P. andrewsi-8 #1351	S.GCTAAATAGT TGCGTGAAAT CTTGTATTTC ACCGCTACTT CTTAGAGGGA
>P. andrewsi-8 #1401	S.CTTTGTGTGT TTAACACAAG GAAGCTTGAG GCAATAACAG GTCTGTGATG
>P. andrewsi-8 #1451	S.CCCTTAGATG TTCTGGGCTG CACGCGCGCT ACACTGACAC GATCAACGAG
>P. andrewsi-8 #1501	TATTTCCTTG CCCGGTAGGG TTAGGGTAAT CTTTTGAAAT CGTGTCGTGC
>P. andrewsi-8 #1551	S.TAGGGATAGA CGATTGCAAT TATTCGTCTT CAACGAGGAA TTCCTAGTAA
#1601	S.ATGCAAGTCA TCAGCTTGCG TTGATTACGT CCCTGCCCTT TGTACACACC
#1651	G.GCCCGTCGCT CCTACCGATT GAGTGATCCG GTGAGCTGTC CGGACTGCGA
#1701	S.TTAGTTCAGT TTCTGTTCTT TTCGCGGGAA GTTCTGCAAA CCTTATCACT
>P. andrewsi-9 #1751	S.TAGAGGAAGG AGAAGTCGTA ACAAGGTTTC CGTAGGTGAA CCTGCAGAAG

FIG. 18B

>P. andrewsi-S.GATCATTC

- ACACCGATTC ATTCTCTGAG AAACCAGCGG TCTCTGTAAA AGGAGATGGG #1
- ATCTCCGCTT TGTTTAGATC CCCACACCTG ACCGCTTTAA CGGGCCGGGT #51
- AGGTGCATAA CTTCTATGAA CCAATTGTAC TAGTCTAAAG TATCCAATAT #101
- CCTTTTGGAT TTTGGTATTT CAAAACGAAA TTCCAAACTC TCAACGATGG #151
- ATGCCTCGGC TCGAGAATCG ATGAAGGACG CAGCGAAGTG CGATAAGCAC #201
- TGCGATTTGC AGAATTCCGT GAACCAGTAG AAATCTCAAC GCATACTGCA #251
- CAAAGGGGAT TTATCCTCTT TGTACATACA TATCAGTGTC GCTCTTCTTC #301
- CCGATACAAA CATTTTGTTG ATTTACAATC AACATTATGC TTTGTATCCC #351
- GCTTGGATTC CTTTATTGGG ATCCGCTGTG TGCGCTTGCT GACACAGGCG #401
- CATTAATTTG CAAGGCTATA ATACTACTGT ACTGTAGCCC CTTCGCAAGA #451
- AGGACTGCGC TAGTGAGTAT CTTTGGATGC TCGCGAACTC GACTGTGTTG #501
- TGGTTGATTC CGTGTTCCTC GATCACGCGA TTCATCGCTT CAACGCATTA #551
- TGTCAAATTT GATGAATGCA GAGAGTTGTT TATGAATTAC GCGATCGCTT #601
- TGGTCTCAGA ATCGTTACTA TAGCACGCTT GTCGGTTTGC AACCTGGCAA #651
- TATGTCATCA TT #701

FIG. 19

						Primers to claim			
Perkinsus species	PCR	Name	Forward Primer (5'-3')	Position ¹	Name	Reverse Primer (5'-3')	Position ¹	Amplicon Size (hn)	Publication
Perkinsus marinus	Species	300F	CAC TTG TAT TGT	08-09	300R	TTG GTG ACA TCT	346-366	307	Marsh et al.
	specific	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	GAA GCA CCC			CCA AAT GAC			J. Parasitol. 1995 81(4):577-83.
									J. Parasitol. 1999 85(4):650-6.
Perkinsus atlanticus Species	Species	PA690F	PA690F ATG CTA TGG TTG	262-283	PA690R	PA690R GTA GCA AGC CGT	933-952	691	Robledo et al.
	specific		GTT GCG GAC C			AGA ACA GC			J. Parasitol. 2000 86(5):972-8
Perkinsus andrewsi ² Species		NTS7		447-470	9SLN	ATT GTG TAA CCA	717-736	290	Coss et al.
	specific		TGG			CCC CAG GC			J. Euk. Microbiol. (In
	*******	*****	AGG CGT GGT			•	***********		Press)
		.,,	GAC						
Perkinsus marinus	Generic	PER1	TAG TAC CCG CTC	827-845	PER2	TGC AAT GCT TGC	1123-1139		
			AT(TC) GTG G		.,,,,,,,,,,	GAG CT			J. Parasitol. (Submitted)
Perkinsus atlanticus Generic	Generic	PER1	TAG TAC CCG CTC	833-851	PER2	TGC AAT GCT TGC	1121-1137	305	Coss et al.
			ATT GTG G			GAG CT			J. Parasitol. (Submitted)
Perkinsus andrewsi Generic	Generic	PER1	TAG TAC CCG CTC	1221-1239	PER2	TGC AAT GCT TGC	1523-1539	319	Coss et al.
			ATT GTG G			GAGCT			J. Parasitol. (Submitted)

'Relative to the NTS sequence

²Perkinsus sp. (Macoma balthica)

FIG. 20

					Primers	Primers to claim		
Perkinsus species PCR		Name	Name Forward Primer (5'-3') Position	Position	Name	Name Forward Primer (5'-3')	Position ¹	Publication
Perkinsus andrewsi Sequencing SSU3F	Sequencing	SSU3F	AGT TGG ATT TCT	626-647	SSU4F A	ACC AGG TCC AGA	1218-1239	Coss et al.
	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	***************************************	GCC TTG GGC G			CAT AGG AAG G		J. Euk. Microbiol. (In Press)

FIG. 21